ANNAL. BIOL. TIHANY | 27 | 193—220 | HUNGARIA 1960

PHOTOSYNTHETIC EXPERIMENTS WITH UNICELLULAR ALGAE OF DIFFERENT PHOTOSYNTHETIC TYPE

LAJOS J. M. FELFÖLDY

Received: 11th March, 1960.

From the plant physiological point of view, the most interesting problem of the primary production of fresh-water bodies is to what extent autotrophic aquatic organisms are able to utilize inorganic carbon sources. With other words: which forms of carbonic acid compounds are available for the different species? It has been established by previous investigations (FELFÖLDY 1960) that certain algal strains can utilize free carbon dioxide only, while in others an intensive carbon uptake was observed also from the 3 mM KOH—K_2CO_3—$KHCO_3$ buffer system at high pH and carbonate ion concentration. These experiments were carried out by RUTTNER's conductometric method. It has been suggested to continue these investigations with more precise methods in order to obtain more accurate quantitative results.

In this paper the results obtained by the usual WARBURG method are discussed.

Materials and methods

In these experiments bacteria-free algal strains were used, which were taken from the algal collection of the Hungarian Biological Research Institute, Tihany, and had been isolated partly from Lake Balaton, partly from garden soil in the neighbourhood of the Institute by ZSUZSA F. KALKÓ.

The strains investigated are as follows:

7K *Chlorella vulgaris* BEYER. Isolated from Lake Balaton, 14. January 1954.

172 *Kirchneriella contorta* (SCHMIDLE) BOHLIN. From Lake Balaton, 29. January 1955.

953 *Coelastrum microporum* NÄG. From Lake Balaton, 19. August 1955.

3153 *Chlorocloster terrestris* PASCHER. From garden soil at Tihany, 6. November 1957.

The bacteria-free cultures are maintained in a northern window on agar slants containing glucose, peptone, yeast hydrolizate and the inorganic salts of the KNOP—PRINGSHEIM nutrient solution (PRINGSHEIM 1946, 35). The algal material used in the experiments was cultured in 2 litre ERLENMEYER flasks containing one litre KNOP—PRINGSHEIM liquid medium. These prepara-

tory cultures were illuminated by incandescent lamps (c. 7000 lux), at 25—30°C temperature and were aerated with 3% carbon dioxide in air. When sufficient amount of cells had developed in the cultures, aliquots were taken from them. The cells in these samples were washed 3-times with, and finally transferred into the experimental solutions, which were prepared as described below.

In the course of these experiments the photosynthetic activity of the above four strains was studied in different media by the usual WARBURG method (WARBURG 1919, GAFFRON 1939).

The measurements were performed in the usual vessels, illuminated by a 40W fluorescent tube (Tungsram F2) built into the apparatus (light intensity c. 5000 lux), at a temperature of $25{,}0 \pm 0{,}5°$ C, and at a shaking velocity of about 110 cycles/min. Each experiment was made with four parallels, 10 ml suspension being pipetted into two vessels and 20 ml into another two. For the determination of dry matter either weighted filter crucible with sintered glass (Jena G4), or Delta filter paper № 368 were used, through which aliquots of the experimental suspensions were filtered and washed with distilled water.

In compliance with the purpose of the experiments, solutions of different carbon dioxide deficiency referred to the total amount of CO_2 present in the 3 mM $KHCO_3$ solution, were prepared. Instead of using the mixtures of 3 mM $KHCO_3$ and K_2CO_3 solutions, a 3 mM KOH solution was made, through which CO_2 gas was blown. Measuring continuously the electrical conductance, solutions of known CO_2 deficiency can be prepared. The calculation of the CO_2 deficiency in a given solution was made partly on the basis of RUTTNER's (1948, 213) nomogram, and partly from the relations existing between pH and the carbonic acid components using ÖSTERLIND's (1949) formulas. The physico-chemical properties of the experimental solutions prepared in the above manner (K_{18} and pH) were always registered before and after each experiment.

The conductivity of the solutions was measured with an electronic instrument "Resistoscope type HRE 13—58" (Híradástechnikai KTSz—Budapest), with platinum electrode of a capacity of C=0,054. The pH of the samples was measured with a "pH-Electrometer type 2512" (Orion—Budapest), with glass and calomel electrodes. The instrument was calibrated in the alkaline range by ATKINS—PANTIN (1926) buffer solution before each series of measurements.

Experimental results

In *Table 1* the properties of the experimental solutions used are tabulated.

Table 1

Physico-chemical properties of the three experimental solutions

No	$K_{18} \cdot 10^4$	pH	mM/litre		
			c_{CO_2}	$c_{HCO_3^-}$	$c_{CO_3^{--}}$
1	2,99	7,02	0,66	2,99	0,002
2	3,13	7,02	$1{,}5.10^{-3}$	2,18	0,39
3	3,88	10,73	$<2{,}4.10^{-5}$	0,33	1,04

The solution № 1 is actually a 3 mM $KHCO_3$ solution containing 0,66 mM/litre carbon dioxide in free form. Its symbol in the figures is "1. CO_2". The solution № 2 is a mixture of $KHCO_3$ and K_2CO_3: solutions, in which the amount of HCO_3^- ions predominate over CO_3^{--} ions. Its symbol is: "2. $KHCO_3$" The maximal CO_3^{--} content in the 3 mM KOH solution through which CO_2-gas is bubbled was reached at pH 10,6 (1,06 mM/litre CO_3^{--}). The pH-value of our solution № 3 is somewhat higher: 10,73 and its HCO_3^- content amounts only to one third of the carbonates present. This solution is marked in the figures with symbol "3. CO_3^{--}".

The data and the results of the experiments are seen in *Figures 1—4* and in their explanations.

Discussion

In the course of experiments three types of photosynthesis were distinguished in the species investigated.

The first type of photosynthesis was found in the case of *Chlorella vulgaris*. In this strain the rate of photosynthesis is the function of the amount of free CO_2 present in the solution. It can be seen in *Figure 1* that the photosynthetic rates in solutions № 1 and № 2 gradually decrease in time. This decrease is slower in solutions containing more carbon dioxide (№ 1). In solution № 3 no release of oxygen was observed. In this solution, containing CO_3^{--} surplus and only negligible amount of dissociated carbon dioxide, O_2 uptake (respiration) was registered only.

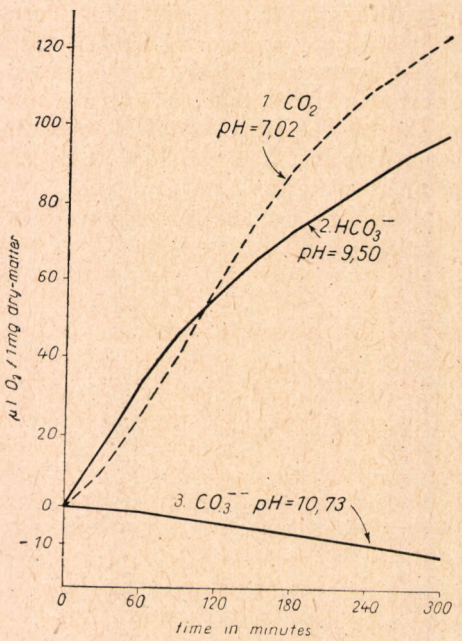
The photosynthesis of *Kirchneriella* belongs to the second type. It photosynthesizes with about the same intensity in all three solutions. In the HCO_3^- and CO_3^{--} solutions the outset of photosynthesis is delayed after the beginning of illumination. This lag-period was not observable in solutions containing free CO_2. After the lag-period the intensity of photosynthesis is but a little greater (42,8 μl O_2/hour in average) in the solution № 2, than in the presence of free CO_2 (40,8 μl O_2/hour in average in the № 1 solution). The photosynthetic rate in solution № 3 is lower than that measured in the other two solutions (36,4 μl O_2/hour) lag-period also lasting longer *(Table 2)*.

A third type of photosynthesis was observed in the case of *Coelastrum (Figure 3)* and *Chlorocloster (Figure 4)*. The difference between this type and the other two is that in solutions containing free carbon dioxide there is

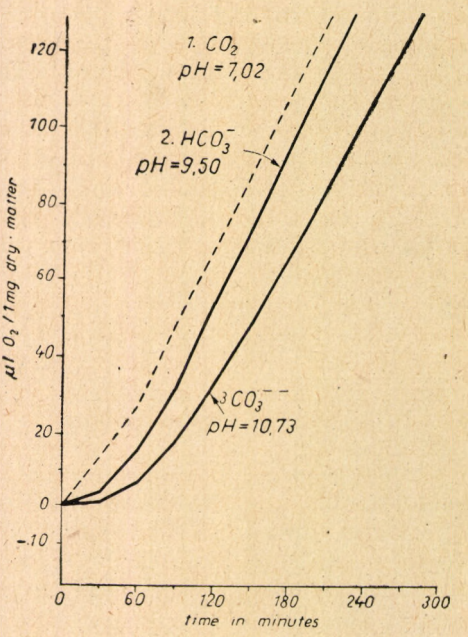
Table 2

Duration of lag-period and average photosynthetic activity (μl O_2/hour) in four strains measured in the three experimental solutions

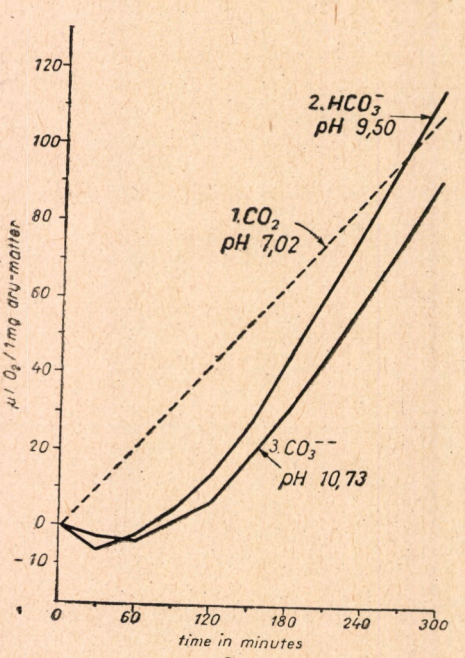
Name of strains	CO_2 μl O_2/hour	HCO_3^-		CO_3^{--}	
		lag-period min.	μl O_2 per hour	lag-period min.	μl O_2 per hour
7K Chlorella	36,8—14,2 gradually slows down	none	34,6—10,2 gradually slows down	none	0,0
172 Kirchneriella	40,8	60	42,8	90	36,4
953 Coelastrum	21,8	150	35,1	150	30,2
3153 Chlorocloster	15,2	180	26,0	60	27,3


1. CO_2 pH=7,02
2. HCO_3^- pH=9,50
3. CO_3^{--} pH=10,73
µl O_2 / 1mg dry-matter
time in minutes

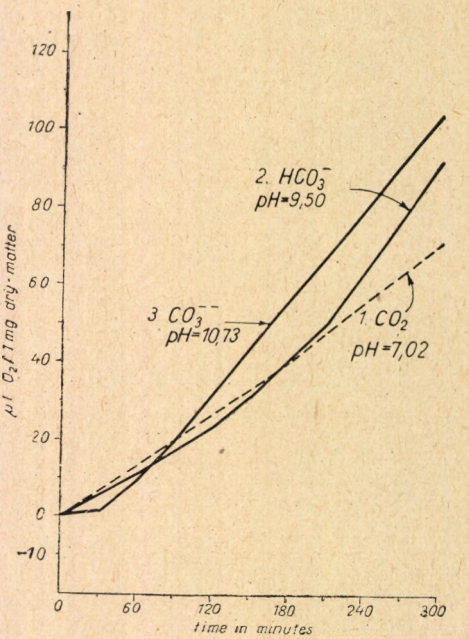
1


1. CO_2 pH=7,02
2. HCO_3^- pH=9,50
3 CO_3^{--} pH=10,73
µl O_2 / 1mg dry · matter
time in minutes

2


2. HCO_3^- pH 9,50
1. CO_2 pH 7,02
3. CO_3^{--} pH 10,73
µl O_2 / 1mg dry-matter
time in minutes

3


2. HCO_3^- pH=9,50
3. CO_3^{--} pH=10,73
1. CO_2 pH=7,02
µl O_2 / 1mg dry · matter
time in minutes

4

no lag-period at the beginning of illumination, but the intensity of photosynthesis is lower than those measured in solutions containing HCO_3^- and CO_3^{--} respectively. In this type the lag-period appears regularly at the beginning of the experiment in solutions containing hydrocarbonates and carbonates. In *Figure 4* the graphs illustrating the photosynthesis in *Chlorocloster*, do not indicate a long lag-period, as the experimental suspensions were left in day- light for a while before the experiment.

In case the suspensions are previously illuminated, the lag-period disappears. If *Coelastrum microporum* cells suspended in solution № 3 are left illuminated at an intensity of 7000 Lux for three hours at a temperature of 25° C, and thereafter transferred into Warburg vessels to measure photosynthetic activity, no lag-period was observed. During an illumination lasting for three hours the cells get accustomed to the alkaline medium and to the uptake or utilization of hydrocarbonate ions. (The expression "adaptation" should be avoided for its definite genetical meaning in microbiology.)

This lag-period was detected by ÖSTERLIND (1951, 1952), who studied it in detail when measuring the rate of growth of his *Scenedesmus quadricauda* strain. In his opinion bicarbonate assimilation is a more complicated process, than carbon dioxide assimilation. CO_2-assimilation always begins immediately when illumination is started not taking into consideration the delay caused by the slow diffusion from water phase to gas phase in the vessels and eventual short time induction phenomena. The bicarbonate assimilation, on the contrary, often begins only after a lag-period of 20—40 minutes (in our experiments it sometimes lasted for as much as 150 minutes), during which period some factor necessary for the bicarbonate assimilation is being activated. He further stated that this activation of algae can occur only in light and that the age of the culture and conditions under which the inoculum was cultured may have a certain effect on photoactivation.

It seems very important, from the hydrobiological point of view, to thoroughly study this activation phenomenon, for it indicates that definite qualitative difference exists between the assimilation of free carbon dioxide and

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Figure 1. Photosynthetic activity of 7K *Chlorella vulgaris* BEYER. in three different solutions. Time of experiment: 12. March 1959. Three weeks old culture, aerated with 3 per cent carbon dioxide in air

1. ábra. 7K *Chlorella vulgaris* fotoszintézise három különféle oldatban

Figure 2. Photosynthetic activity of 172 *Kirchneriella contorta* (SCHMIDLE) BOHLIN in three solutions of different carbon content. Time of experiment: 6. March 1959. The preparatory culture is two weeks old, aerated with 3 per cent carbon dioxide in air

2. ábra. 172. *Kirchneriella contorta* (SCHMIDLE) BOHLIN fotoszintézise három különböző széntartalmú oldatban

Figure 3. Photosynthetic rates of 953 *Coelastrum microporum* NÄG. in three solutions represented in *Table 1.* Time of experiment: 3. May 1959. Four weeks old preparatory culture, aerated with 3 per cent carbon dioxide in air

3. ábra. 953. *Coelastrum microporum* NÄG. fotoszintézise három különböző oldatban

Figure 4. Photosynthetic curves of 3153 *Chlorocloster terrestris* PASCHER in three solutions showing different ratios of the various carbonic acid components *(Table 1)*. Date of experiment: 13 March 1959. Three and half weeks old preparatory culture aerated with 3 per cent CO_2 in air

4. ábra. 3153. *Chlorocloster terrestris* PASCHER fotoszintézise három különböző oldatban

that of hydrocarbonate (or probably carbonate) ions. This lag-period reminds us further that the ability of planktonic algae to use hydrocarbonate ions is the results of a "training" taking place in time. The fact that conditions under which preparatory cultures are grown may have an important effect on the utilization of inorganic carbon sources by unicellular algae, reminds us not to draw premature conclusions when evaluating physiological experiments of such nature.

The discussion thus far appears to strengthen the inference that strains, which show a more intensive photosynthesis in solution № 3 than in solution № 2 (for instance *Chlorocloster), i.e.* their photosynthesis is intensive at pH 10,7, at the presence of three times as much carbonate than hydrocarbonate ions, are presumably capable of the uptake of carbonate ions. This question necessitates further investigations.

It is very difficult to decide, whether the effect of pH or the availability of the carbonic acid components are responsible for the differences observed, for it may be easily understood that it is not possible to investigate the growth or assimilation of an autotrophic aquatic organism at various pH values, without taking into consideration some changes occurring in the forms of carbonic acid compounds.

Up to now, no reference was made in the literature of algal physiology to algal strains which photosynthesize in media of such high pH as was used in our experiments (Wann and Hopkins 1927, Emerson and Green 1938, Pratt 1943, Österlind 1951a, Steemann Nielsen 1952, 1955, Bierhuizen 1957, Lorenzen 1958). Only Österlind (1948, 1949) informs us of a *Scenedesmus quadricauda* strain with growth-optimum at pH 8,1.

Most algae in our collection of algal strains were isolated partly from Lake Balaton (pH about 8,4; carbonate content 1—6 mg/litre), partly from pond "Belső tó" at Tihany (pH about 8,8; carbonate content 50—150 mg/litre) (Entz 1951) and it may be assumed that these strains had been trained already in their natural habitats to the high pH and to the presence of carbonate ions.

Summary

Photosynthetic curves of four algal strains (7K *Chlorella vulgaris* Beyer . , 172 *Kirchneriella contorta* (Schmidle) Bohlin, 953 *Coelastrum microporum* Näg., 3153 *Chlorocloster terrestris* Pascher.) investigated in a 3 mM KOH—K_2CO_3—$KHCO_3$ buffer system of different CO_2-deficiency by the usual Warburg method are discussed in this paper. On the basis of these experiments three photosynthetic types can be distinguished.

In the first type only free carbon dioxide is utilized *(Chlorella vulgaris),* and photosynthesis decreases gradually parallel with the diminution of CO_2 content. There is no photosynthesis at high pH values and at a surplus of carbonate ions.

The strain belonging to the second type can utilize both free carbon dioxide and hydrocarbonate ions, but its rate of photosynthesis becomes slower in a more alkaline carbonate-containing medium *(Kirchneriella).*

The algae belonging to the third type can photosynthesize, after a certain lag-period, most vigorously in a solution containing hydrocarbonates and a surplus of carbonates. Their photosynthesis in solutions containing

free carbon dioxide, though starting just at the beginning of illumination, is slower than in buffers containing either hydrocarbonates or carbonates (*Coelastrum* and *Chlorocloster*).

The experiments presented seem to support the hypothesis that the strains exhibiting a greater photosynthetic activity in solutions where carbonate ions predominate over hydrocarbonate ions are able to utilize the carbonate ions too. It is still undecided, whether the differences in photosynthetic rates in various buffer systems depend on the availability of carbon sources or on hydrogen ion concentration.

The great pH tolerance in alkaline range of the strains investigated may perhaps be brought into connection with the chemical properties of their natural habitats.

It seems to be an important assumption from the hydrobiological point of view that the ability of using hydrocarbonate (or carbonate) ions by planktonic algae is the result of a "training" taking place in a certain space of time. Thus, the main purpose of physiological research, when dealing with the ecology of photosynthesis in planktonic algae, should be to study the aptitude for this adaptation in the different species.

It is a pleasure to acknowledge the English translation and helpful assistance in experiments of Mrs. Zsuzsa F. Kalkó. I should like to thank Mr. Sándor Herodek for fruitful discussions on some mathematical and physico-chemical problems.

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EGYSEJTŰ ALGÁK KÜLÖNFÉLE FOTOSZINTÉZIS-TÍPUSÁRÓL

Felföldy Lajos

Összefoglalás

Intézetünk élő alga-tenyészet gyűjteményének négy baktérium-mentes törzsével (7K *Chlorella vulgaris* BEYER., 172. *Kirchneriella contorta* (SCHMIDLE) BOHLIN, 953. *Coelastrum microporum* NÄG. és 3153. *Chlorocloster terrestris* PASCHER) végeztünk fotoszintézis-méréseket különféle szén-tartalmú 3 mM KOH-K_2CO_3-$KHCO_3$ puffer elegyekben *(1. táblázat)* WARBURG manometriás módszerével. A kísérletek alapján három, egymástól lényegesen különböző fotoszintézis-típust különböztethettünk meg.

Az első, az elsősorban szabad széndioxidot hasznosító típus *(Chlorella)*, melynek fotoszintézis-intenzitása a szabad CO_2 jelenlététől függ: annak csökkenésével párhuzamosan az asszimiláció is fokozatosan lassul, nagy pH és karbonát-ionok túlsúlya esetén egyáltalán nem folyik *(1. ábra)*.

A második típushoz a szabad széndioxid és karbonát-ionok jelenlétében egyformán, meglehetősen hasonló nagyságrendű gyorsasággal fotoszintetizáló *Kirchneriella* tartozik, mely azonban karbonát-tartalmú közegben dolgozik leglassabban. Hidrokarbonát és karbonát-tartalmú oldatokban az O_2-leadás határozott késéssel indul (lappangási időszak) *(2. ábra)*.

A harmadik típus esetében ez a lappangás még markánsabb a HCO_3^- illetve CO_3^{--}-tartalmú közegekben, melyekben a fotoszintézis tempója a kezdeti késés után nagyobb, mint a széndioxid jelenlétében mért aktivitás. CO_2-tartalmú oldatban a lappangási időszak hiányzik *(Coelastrum* és *Chlorocloster) (3.* és *4. ábra)*.

Azoknak a törzseknek esetében, melyek a karbonát tartalmú oldatban intenzívebben asszimilálnak, mint a kevésbé lúgosakban, fennáll a karbonát-ionok hasznosításának lehetősége is, de a kérdés további kísérletezést igényel. Azt sem sikerült eddig eldönteni, hogy a kapott különbségek a szervetlen szénforrás hozzáférhetőségén, vagy inkább a hidrogén-ion koncentráció változásain alapulnak-e?

Vizsgált algáink pH- és karbonát-ion tűrése valószínűleg természetes termőhelyük viszonyaival hozható összefüggésbe: a Balatonban (pH 8,4 körül, 1—6 mg CO_3^{--}/liter) és a tihanyi Belső-tóban (pH 8,8 körül, 50—150 mg CO_3^{--}/liter) hozzászokhattak a lúgos miliőhöz és a karbonát-ionok jelenlétéhez. Ez a tény magyarázhatja meg eredményeink eltérő voltát az eddigi — elsősorban *Chlorella* és *Scenedesmus* fajokkal végzett kísérleteken alapuló — közleményektől.

Hidrobiológiai szempontból a lappangási periódus kutatása látszik különösen fontosnak, mert az egyrészt a szabad CO_2 és a hidrokarbonát-ion asszimilációja közti minőségi különbségre mutat rá, másrészt figyelmeztet, hogy a hidrokarbonát (és esetleg karbonát) ionok kihasználási lehetősége időben lejátszódó hozzászokás eredménye a planktonalgák esetében, tehát az ilyen irányú alga-élettani munka helyes problémafelvetése az, hogy a különböző fajok és törzsek képesek-e erre az alkalmazkodásra vagy nem?